Remarks

In accordance with present invention, there are provided chimeric proteins comprising at least two functional protein units, wherein each functional unit comprises the dimerization domain of a member of the steroid/thyroid hormone nuclear receptor superfamily. When these two protein units associate, a functional entity is formed, for example, a functional heterodimer or homodimer. Interestingly, many of the invention chimeric protein dimers display functional properties that are distinct from wild type dimers of members of the superfamily, for example, DNA binding superior to that of wild type complexes. Thus, the chimeric proteins of the invention can be used in a variety of methods to analyze and modulate gene expression in cells and organisms.

Claims 1-60 were pending before this communication. Claims 23-51 and 55-60 have been withdrawn from consideration pursuant to the election of Group I with traverse. By this response, claim 18 has been amended and claim 12 has been cancelled to correct obvious typographical errors. These amendments add no new matter as they are fully supported by the specification and the original claims. Attached hereto is a marked-up version of the changes made to the claims, labeled APPENDIX A.

Accordingly, claims 1-11, 13-22 and 52-54 are currently under consideration. For the Examiner's convenience, a clean copy of these claims is also provided in APPENDIX B. Claims 1-11 and 13-60 remain pending in this application.

The rejection of claims 1-22 and 52-54 under 35 U.S.C. § 112, first paragraph, because the specification allegedly fails to reasonably provide enablement for the chimeric proteins as claimed is respectfully traversed. It is respectfully submitted that the specification fully enables one of skill in the art to make and use the invention and is commensurate with the scope of the claims.

In fact, the Examiner has acknowledged that the specification is enabling for a chimeric protein wherein the dimerization domain is obtained from the ecdysone receptor, a Usp receptor or a retinoid X receptor (see Office Action, Paper No. 10, page 3, lines 7-11). It is respectfully submitted, however, that that the claims should not be limited to just the working examples provided because the superfamily member examples provided (i.e., ecdysone, Usp and RXR), are highly representative of the entire superfamily. Thus, additional examples with further superfamily members are clearly not necessary. Indeed, given the well characterized nature of all members of the nuclear receptor superfamily, additional examples with further superfamily members would merely be superfluous.

In addition, the Examiner's efforts to unduly limit the functional properties which characterize the claimed chimeric proteins by requiring that a chimeric protein "respond to application of hormone" (see Office Action, Paper No. 10, at pages 3-4) is respectfully submitted to be misplaced. Contrary to the Examiner's assertion, ligand or hormone binding by a claimed chimeric protein is only one measure of its function. The present invention, as defined by claim 1, requires the two functional protein units of the claimed chimeric protein to form a functional entity. The specification clearly defines a functional dimer or a functional entity as possessing at least some of the biological function of a dimer formed between two equivalent monomeric species (see, for example, specification at page 11, lines 26-30). "The biological function of such dimers includes one or more of the following properties: DNA binding, ligand binding, transactivation, and dimerization properties related to transactivation of a promoter operatively associated with a response element responsive to the invention chimeric protein" (emphasis added, see specification at page 12, lines 1-4). Therefore, the ligand binding properties of a chimeric dimer are only one of the biological functions which invention chimeric proteins may possess (see also, specification at page 23, lines 23-29).

Moreover, it is clear from the specification that the invention contemplates more than function monitored by ligand binding.

> It is specifically contemplated within the scope of the present invention that modulation includes repression of expression of one or more genes [and s]uch repression can be either ligand-dependent repression or repression that occurs independently of the presence of a ligand. Thus, there are four types of modulation contemplated within the scope of the invention: ligand-dependent induced modulation, ligand-dependent repressed modulation, ligand-independent induced modulation and ligand-independent repressed modulation.

(emphasis added, see specification at page 23, lines 23-29). Therefore, function is not solely defined by ligand binding, and should not define the scope of enablement.

Therefore, it is respectfully submitted that an appropriate analysis of enablement must include consideration of all of the functions of the chimeric protein contemplated in the specification, i.e., DNA binding, ligand binding, transactivation, and dimerization properties related to transactivation. Indeed, many of the invention functional dimers display DNA binding equivalent or superior to that of wild type dimers; and certain dimers lose the capacity to transactivate and, as a result, function like constitutive repressors (see, for example, specification at page 5, lines 14-21). Each of these functions facilitate the use of novel chimeric proteins to regulate target exogenous genes. Accordingly, the specification clearly enables one of skill in the art to make and use a chimeric protein that comprises two functional protein units that form a functional entity, not just a ligand binding entity.

The standard for determining enablement is whether the specification as filed provides sufficient information so as to permit one skilled in the art to make and use the claimed invention (*United States v. Telectronics, Inc.*, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988)). The test of enablement is not whether experimentation is necessary, but rather whether any experimentation that is necessary is undue. *Id.* "[A] considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation would proceed" (*In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)).

The factors set forth in *In re Wands* as applied to the present invention, when considering the full spectrum of the contemplated biological functions of the chimeric proteins, clearly show that the present disclosure is enabling. The following Wands factors (as numbered in the Office Action, Paper No. 10), are of particular note.

(Wands Factor 1) The breadth of the claims is commensurate with the disclosure in light of the relationship between members of the steroid/thyroid hormone nuclear receptor superfamily.

Applicants respectfully disagree with the Examiner's assertion that claim 1 is allegedly overly broad "since the Specification provides insufficient guidance as to which of the myriad of fusion polypeptides encompassed by the claim will retain the functional characteristics such that it can be used as a non-mammalian based transcription regulating system . . ." (see Office Action, Paper No. 10, at page 3 lines, 17-20).

The present invention, as defined by claim 1, encompasses chimeric proteins formed from protein units derived from a member of the steroid/thyroid hormone nuclear receptor superfamily (see, for example, specification at page 13, line 18 through page 14, line 11). Members of the superfamily are related by structure and function, and they are all, by definition, hormone binding proteins that operate as transcription factors (see, for example, specification at page 13, lines 12-17).

In particular, invention chimeric proteins comprise the dimerization domain of two members of the superfamily. Members of the superfamily are commonly characterized by the presence of five domains, one of which is this dimerization domain (see, for example, specification at page 11, lines 3-7); and significant homologies exist in domains within members of the superfamily, which have been extensively studied in the art. For example, Usp and RXR share a significant degree of sequence homology which allows for dimerization between EcR (the natural dimer for Usp) and RXR (see, for example, specification at page 3, lines 11-26). This exemplifies the interchangeability of dimerization domains of different members of the superfamily to create novel functional chimeric protein dimers.

Therefore, because the instant specification "discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim," the enablement requirement of 35 U.S.C. § 112 is satisfied (*In re Fisher*, 166 USPQ 18, 24 (CCPA 1970); MPEP § 2164.01(b)). The receptors used in the examples provided (i.e., the ecdysone, Usp or retinoid X receptors) are highly representative of the superfamily. Accordingly, Applicants are entitled to claims representing all members of the steroid/thyroid hormone nuclear receptor superfamily.

(Wands Factor 6) The amount of direction or guidance provided by the inventor in the specification is extensive; and (Wands Factor 7) Numerous and thorough working examples are provided.

Applicants respectfully disagree with the Examiner's assertion that "[t]here is insufficient guidance provided in the specification as to how one of ordinary skill in the art would generate a chimeric protein . . . other than those exemplified in the specification . . ." (see Office Action, Paper No. 10, at page 4, lines 6-10). Contrary to the Examiner's assertion, the specification clearly provides extensive direction for making and using the invention chimeric proteins.

For example, the design of a functional dimer is taught in Example 1; the construction of a fusion protein containing such a functional dimer is taught in Example 1; and the subsequent transfection of the fusion protein constructs is taught in Example 2. A variety of functional analyses are also provided in the working examples. For example, dimerization can be detected by gel mobility shift analysis or Western blot analysis as taught in Example 3; ligand binding and transactivation can be detected by reporter constructs as taught in Example 2; and DNA-binding can be detected by gel mobility shift analysis with labeled target DNA as taught in Example 3. Due to the functional and structural similarity of the members of the steroid/thyroid hormone nuclear receptor superfamily, any members can be used to create the chimeric proteins of the present invention following the guidance provided.

Therefore, when considering all of the contemplated functions which may be exhibited by the claimed chimeric protein, the specification clearly enables one of skill in the art to make and use these claimed chimeric proteins.

(Wands Factor 5) The level of predictability in the art is relatively high when all possible functional entities are considered as discussed above.

Applicants further disagree with the Examiner's assertion that the "results of making chimeric proteins comprising regions from these receptors are unpredictable" (see Office Action, Paper No. 10, page 4, lines 1-2). It is respectfully submitted that one of skill in the art could reliably identify dimerization domains with which to form a functional entity (i.e., an entity capable of the function of dimerization in this example) based on structural and sequence homology and the conserved domain structure of members of the steroid/thyroid hormone nuclear receptor superfamily. Therefore, the creation of a functional entity comprising two protein units containing such dimerization domains is highly predictable.

To the contrary, the Examiner has improperly concluded that the results are unpredictable based on the fact that certain chimeric proteins may or may not respond to the application of ligand (see Office Action, Paper No. 10, page 4, lines 2-6). As noted above, ligand binding may or may not be altered in a chimeric protein of the present invention. However, there are several other functional parameters that can simply be determined and applied to make and use a functional entity as required by following the teachings of the specification.

Additional Wands factors

The state of the prior art is such that one of ordinary skill in the art would readily be able to make and use the invention as taught (Wands Factor 3); the level of one of ordinary skill of those in the relevant art is high (Wands Factor 4); and the quantity of experimentation needed to make or use the invention does not constitute undue experimentation because it entails routine methods known to those skilled in the art (Wands Factor 8).

In summary, in view of all of the Wands factors combined, the disclosure is clearly enabling.

For all of the reasons set forth above, it is respectfully submitted that the present claims are fully enabled as required by 35 U.S.C. § 112, first paragraph. Moreover, it is respectfully submitted to be clear that those skilled in the art would not require undue experimentation to practice the claimed invention. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. 112, first paragraph, are respectfully requested.

Conclusion

In view of the above amendments and remarks, reconsideration and favorable action on all claims are respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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Enclosures: Appendices A and B

APPENDIX A – ALTERED CLAIMS

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claim 18 has been amended as follows:

18. The chimeric protein according to claim 15 wherein [~~the chimeric protein is a chimeric protein and~~] the linker comprises the amino acid sequence of SEQ ID NO:15.

Claim 12 has been cancelled.

APPENDIX B – CLAIMS CURRENTLY UNDER CONSIDERATION

1. A chimeric protein comprising:

at least two functional protein units, wherein each functional protein unit comprises the dimerization domain of a member of the steroid/thyroid hormone nuclear receptor superfamily, and

an optional linker interposed therebetween,

wherein the at least two protein units form a functional entity.

2. The chimeric protein according to claim 1 wherein the entity is an endodimer.

3. The chimeric protein according to claim 1 wherein each protein unit comprises a ligand binding domain, an optional hinge domain, and an optional DNA binding domain.

4. The chimeric protein according to claim 3 wherein the functional entity is an endodimer.

5. The chimeric protein according to claim 1 wherein at least one member is non-mammalian.

6. The chimeric protein according to claim 5 wherein the at least one member is from an insect species.

7. The chimeric protein according to claim 1 wherein at least one functional protein unit comprises the dimerization domain of an ecdysone receptor.

8. The chimeric protein according to claim 7 wherein the ecdysone receptor comprises the dimerization domain of a *Drosophila* ecdysone receptor.

9. The chimeric protein according to claim 7 wherein the ecdysone receptor comprises the dimerization domain of a Lepidoptera ecdysone receptor.

10. The chimeric protein according to claim 7 wherein the ecdysone receptor comprises the dimerization domain of a *Bombyx* ecdysone receptor.

11. The chimeric protein according to claim 5 wherein at least one functional protein unit comprises the dimerization domain of the *ultraspiracle* protein.

13. The chimeric protein according to claim 1 wherein at least one functional protein unit comprises the dimerization domain of the retinoid X receptor.

14. The chimeric protein according to claim 1 wherein the protein units are independently selected from the group consisting of glucocorticoid receptors, mineralocorticoid receptors, estrogen receptors, progesterone receptors, androgen receptors, Vitamin D3 receptors, retinoic acid receptors, retinoid X receptors, peroxisome proliferator-activated receptors, thyroid hormone receptors, and steroid and xenobiotic receptors, farnesoid X receptor, pregnenolone X receptor, liver X receptor, and BXR.

15. The chimeric protein according to claim 1 wherein the linker contains from about 5 to about 245 amino acids.

16. The chimeric protein according to claim 15 wherein the linker contains from about 53 to about 125 amino acids.

17. The chimeric protein according to claim 15 wherein the linker comprises glycine, proline, serine, alanine and threonine residues.

18. (Amended) The chimeric protein according to claim 15 wherein the linker comprises the amino acid sequence of SEQ ID NO:15.

19. The chimeric protein according to claim 3 wherein one or more protein units further comprise a C-terminal domain.

20. The chimeric protein according to claim 3 wherein the DNA binding domains of one or more protein units comprise 66 to 68 amino acids, including 9 cysteines.

21. The chimeric protein according to claim 3 wherein the hinge domain of one or more protein units is the *Bombyx* hinge domain.

22. The chimeric protein according to claim 1 wherein one or more protein units further comprise an activation domain.

52. An isolated protein crystal suitable for x-ray diffraction analysis comprising a purified chimeric protein according to claim 1.

53. The protein crystal according to claim 52 further comprising a ligand bound to the purified chimeric protein so as to form a chimeric protein-ligand complex.

54. The protein crystal according to claim 53 further comprising a nucleic acid construct being a putative response element for the complex.